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SEASONAL OCCURRENCE OF XYLEM EMBOLISM IN SUGAR MAPLE (ACER SACCHARUM)[1]

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ABSTRACT

Xylem embolism, the reduction of water flow by air-filled vessels, was measured in a stand of 5- to 8-year-old sugar maple (*Acer saccharum* Marsh.) saplings growing in a nursery bed in northwestern Vermont. Embolism was quantified as percentage loss in hydraulic conductivity of trunk and branch segments relative to maximum values obtained by removing air from vessels by repeated high pressure (173 kPa) perfusions. Ten segments per tree were cut from 6 trees for each of 11 measurement periods spaced at roughly monthly intervals from May 1986 to June 1987. During the 1986 growing season, embolism increased significantly from 11 to 31% in the larger branches and trunk (segment diameter ≥ 0.5 cm), but remained at about 10% in twigs (segment diameter < 0.5 cm). This was unexpected because the greatest water stess and thus potential for embolism occurs in twigs. During the winter, embolism increased throughout the trees and the trend with diameter was reversed; by February, small twigs were 84% embolized vs. 69% for larger branches and trunk. Dye perfusions showed that winter embolism in trunks was localized on the south side; this may have resulted from water loss by sublimation or evaporation in the absence of water uptake. Beginning in late March, embolism decreased throughout the trees to approximately 20% in June. This decrease was associated with positive xylem pressure of at least 16 kPa which may have originated in the roots, because weather conditions at the time were unfavorable for the generation of stem pressures characteristic of *Acer* species in early spring.

IN THE MEDICAL SCIENCES, *embolism* is the blockage of a blood vessel caused by a foreign particle or *embolus*. By analogy, the term can be applied to blockage of water flow in vessels and tracheids of plants; in this case the embolus is an air bubble. These bubbles can arise from air forced out of solution during freezing of xylem water, or by an unknown mechanism associated with the characteristically negative pressure of the transpiration stream. There is evidence suggesting that air is sucked into vessels from neighboring air spaces via pores in the vessel wall (Zimmermann, 1983; Crombie, Hipkins, and Milburn, 1985; Sperry, Holbrook, and Tyree, 1987). Once an air bubble is inside a vessel, negative pressure causes it to expand and block water flow.

There has undoubtedly been selective pressure to minimize xylem embolism, because if unchecked it would block water supply to foliage. Certainly the xylem is well designed in this regard; instead of containing a single open pipe which would conduct water with the least resistance but would become embolized by a single bubble, xylem consists of individual tracheids and vessels with intervascular pit membranes that prevent internal air bubbles from expanding beyond their walls. The smaller the individual conducting unit, the less blockage will be caused by an air bubble arising within. Numerous other adaptations have been proposed, including Zimmermann's "segmentation" concept (Zimmermann, 1983), wherein embolism preferentially occurs in the more water-stressed peripheral parts of plants thereby protecting the proximal parts. Despite the fact that plants have obviously been successful in preventing excessive embolism, we do not know to what extent it occurs and whether it is a factor in their ecophysiology, particularly under conditons of water and freezing stress.

In the present investigation, we have quantified embolism for a year in sugar maple saplings growing in a nursery bed in northwestern Vermont. Our objective was to determine the extent and distribution of embolism that occurred during the growing season induced by water stress, and during the winter when freezing would be expected to create emboli. We also wished to determine if and when these trees reversed embolism and whether this was associated with positive xylem pressures which could dissolve the air bubbles. In sugar maple there are at least two sources of positive pressure: 1) the root pressure phenomenon common to many plants; and 2) stem pressure which

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is characteristic of maples and is activated by freeze-thaw cycles. Stem pressure is considered responsible for the flow of sap in early spring which can be collected for maple syrup (Marvin and Green, 1951).

MATERIALS AND METHODS—*Plant material, sampling, and preparation*—Trees were 2–3 m in height and 5–8 years old, and growing in a bed of sandy soil at the state nursery in Essex Junction, Vermont. Six trees were harvested, one each on successive workday mornings for each of 11 measurement periods spaced at roughly monthly intervals between late May 1986 and mid-June 1987. When trees were in leaf, as many leaves as could be reached were snipped in order to minimize dehydration of the tree after cutting it at ground level. The tree was transferred quickly to a container of water and the remaining foliage removed. In the laboratory, the tree was drawn to scale, and 10 segments 10–20 cm in length were located throughout the tree including main axis and lateral branches. In a large sink, the segments were cut from the sapling taking care that all cuts were made under water and that the basalmost segment was far enough from the original cut end to insure that air had not in this way entered any of the vessels of the segment. Segments were fitted with rubber gaskets made from stoppers or tubing, trimmed with a sharp razor blade, and attached to a solution-filled tubing system designed to measure hydraulic conductivity and embolism. During these operations care was taken to minimize the time segments were out of water.

Measurement of embolism—Embolism was quantified by the percentage increase in hydraulic conductivity of the segments after the removal of air emboli by repeated high-pressure flushes. This method has been used successfully on other species including palms, grapevines, and conifers (Sperry, 1986; Tyree and Dixon, 1986; Sperry et al., 1987), and is reported in detail in Sperry, Donnelly, and Tyree (1988). The ten segments from a tree were attached in parallel to influx and efflux tubing manifolds with stopcocks allowing flow of solution to any or all segments. We used either 0.01 M oxalic acid (pH <2.5) or 0.05% formaldehyde as our solution in order to prevent microbial growth; particulate clogging was reduced by prefiltering to 0.22 μm. For the initial conductivity reading, flow generated by a hydraulic head of 3–10 kPa was directed through each segment in succession while volume flow rate of solution was measured gravimetrically by routing the outlet of the efflux manifold to a reservoir on an electronic balance. A computer in communication with the balance was programmed to output hydraulic conductivity computed as mass flow rate divided by pressure gradient across the segment.

After initial conductivity was measured on 5 of the 10 segments, these first 5 were flushed at approximately 175 kPa with solution while the second 5 were measured; after this the first 5 were measured again and the second 5 flushed. Repeated cycles of measuring and flushing were continued until the conductivity of the segments reached a plateau and no longer increased significantly (as determined by a t test, $P = 0.05$). This could take as little as two, or as many as seven flushes. The initial as a percentage of the maximum conductivity indicated the degree of embolism caused by air blockage in the segment. We expressed this as the "percentage loss in conductivity." This method is supported by preliminary work (Sperry et al., 1988) that indicates flushing dissolves air in the vessels. The elevated pressure is necessary, because emboli will not dissolve in stems soaked in water overnight. This means that for sugar maple, the time between cutting the segments underwater and the completion of the initial conductivity measurement does not influence the results.

Dye perfusion—Embolism on an anatomical scale was studied by making dye perfusions of axis segments adjacent to those used for conductivity measurements, or on separate trees. Segments were prepared as if for conductivity measurements, but fitted in parallel at one end only to a water-filled tubing manifold. The free ends of the segments were immersed in 0.1% safranin dye filtered to 22 μm. The outlet from the manifold was positioned approximately 1 m below the surface of the dye creating a small suction force for dye flow through the segments. Uptake continued for at least 25 min before the dye solution was replaced with tap water for an additional 10 min. The stems were sectioned on a sliding microtome for analysis of dye-stained, functional vessels vs. nonstained, embolized vessels. Control perfusions were made on segments which had been flushed to 100% conductivity in order to determine if the vessels of stems with no embolism were completely stained.

Xylem pressure measurements—During the 1986 growing season, midday and diurnal leaf water potentials of the saplings were periodically measured with the pressure bomb. Positive pressures in the xylem were measured on an occasional basis using bubble manometers

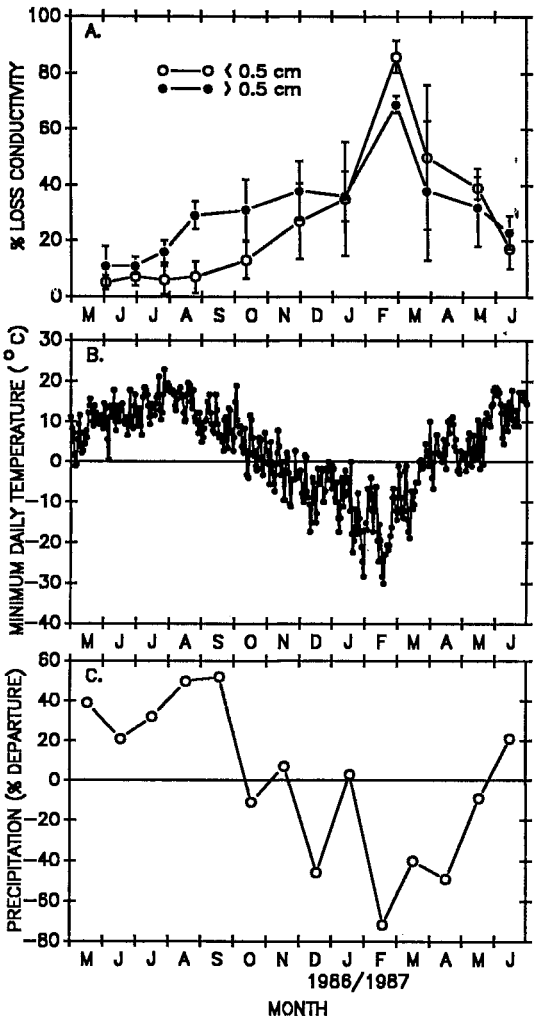

Fig. 1. A. Percentage loss in hydraulic conductivity vs. month beginning May 1986 for segments ≥0.5 cm (solid circles), and <0.5 cm diam (open circles). Means for 6 trees are shown with 95% confidence intervals. B. Minimum daily temperature during study period. C. Monthly precipitation as percentage departure from normal during study period. (Weather data from National Weather Service Office within 3.5 km of study site.)

clamped to lateral branch stubs, or stumps of recently cut trees.

RESULTS—Figure 1A shows the average percentage loss in conductivity, which we synonymize with embolism, for small (<0.5 cm diameter) and large (≥0.5 cm diameter) segments. The saplings had well-defined main axes supporting lateral branches over much of their length. The smaller size class represents the peripheral twigs of the tree and the tip of the main axis; the larger size class are the base of major lateral branches and most of the main axis. The largest diameters were approximately 2 cm for the basal-most main axis segment of the larger trees.

During the 1986 growing season from leaf flush in late May to late August, embolism in the larger size class increased significantly from 11 to 31%, whereas smaller diameter segments remained unchanged near 10%. The tendency for larger diameter segments to have more embolism was also evident in main axis segments in August (Fig. 2A). There was minimal water stress during the growing season of 1986 with midday leaf water potentials rarely dropping below −1.5 MPa; precipitation was above normal throughout the period (Fig. 1C).

With the onset of cold midwinter weather, embolism increased in segments of all sizes seemingly in response to progressively lower minimum temperatures (Fig. 1A, B). Between the January and February measurements, there were 5 days with temperatures below −18 C, with a minimum of −28 C. These low temperatures were associated with a sharp increase in embolism to 69% for larger segments and 89% for small twigs. In contrast to summer conditions, winter embolism was most severe in smaller diameter segments. This was also evident within the main axis (Fig. 2B).

Dye perfusions of trees in winter showed that, beginning with the November measurement, embolism in the main axis was located almost exclusively in the peripheral xylem on the south side. As winter progressed, the area of dysfunction increased in some trees to the entire southern half of the axis from pith to cambium (Fig. 3). Longitudinal sections of segments made on a sliding microtome immediately after perfusion and kept wet throughout the sectioning procedure showed conclusively that vessels in the unstained xylem were air-filled, vs. water-filled in the stained portion (Fig. 5). Twigs also showed one-sided distribution of embolism (Fig. 4a), but it was difficult to demonstrate any correlation with compass direction given their nonvertical orientation. The extreme degree of embolism in twigs in February is best appreciated by dye perfusions shown in Fig. 4a; here only a trace, or no dye at all, was conducted through the twigs. Neighboring twig segments flushed to maximum conductivity and perfused show complete staining (Fig 4b), indicating the effectiveness of the flush in reversing embolism.

To determine if the high levels of embolism in late winter were particular to open-grown sugar maples such as those in the nursery bed where at least the upper soil was frozen, we

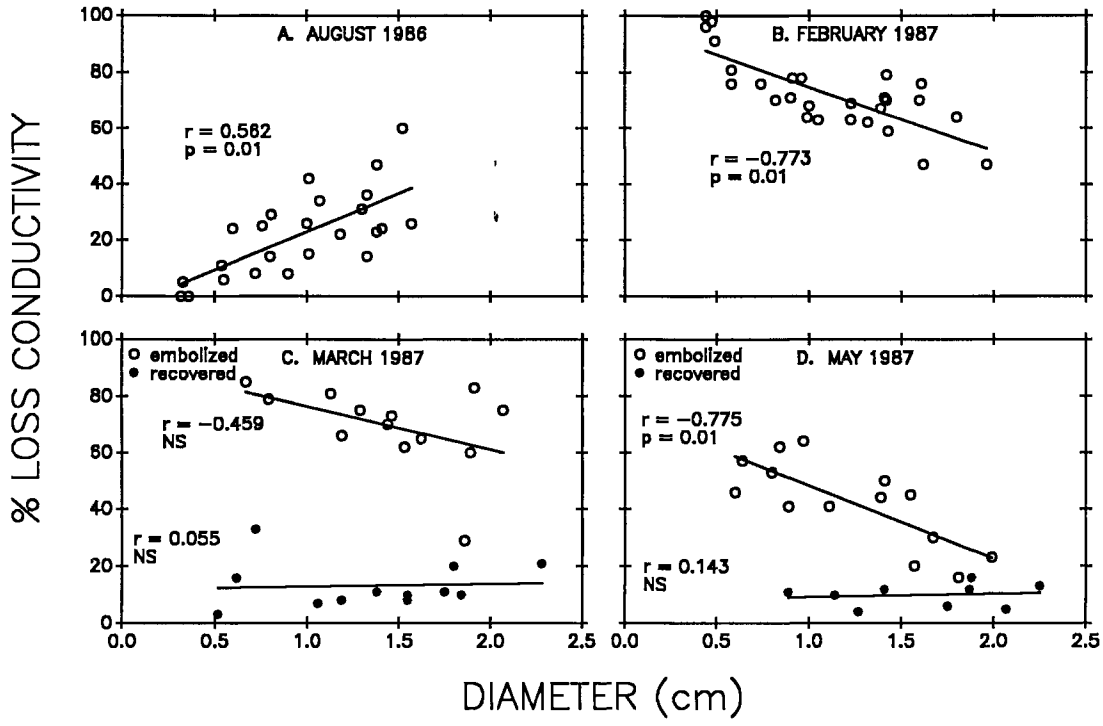

Fig. 2. A-D. Percentage loss in hydraulic conductivity of main axis segments vs. diameter for months indicated; data are pooled from 6 trees. The correlation coefficient r is indicated followed by "NS" if not significant ($P > 0.05$), or the probablility if significant. In March and May, some trees with a higher percent loss conductivity are termed "embolized," vs. "recovered" trees with low loss in conductivity.

sampled saplings in the forest understory where the soil was not frozen. Although embolism was not as extreme as at the nursery site, segments from forest trees were still over half embolized with twigs <0.5 cm diameter (69% loss conductivity) suffering more embolism than larger segments (54% loss conductivity). Dye perfusions showed the same one-sided embolism.

Beginning with the March measurement, embolism decreased to a level in June 1987 nearly equal to that of the previous June (Fig. 1A). Figure 6A shows the daily progress of the March measurement; remarkably, we seem to have caught the trees in the process of embolism reversal. The main-axis embolism of the first two trees (Fig. 6A, 26 and 27 March), plotted as open circles in Fig. 2C (embolized) is indistinguishable from those for February trees in Fig. 2B. These trees contrast with the final three which collectively averaged only 13% loss in conductivity (Fig. 6A, 31 March, 1 and 2 April); these are plotted as solid circles (recovered) in Fig. 2C. In view of these last trees of the March measurement, it was surprising to find that the May averages for embolism were between 30 and 40% (Fig. 1A). Although three of the trees in May did have low embolism values (Fig. 2D, recovered), the other three had much higher values (Fig. 2D, embolized). Apparently some trees were more successful than others in recovering from embolism.

Dye perfusions of recovered vs. nonrecovered trees in May revealed that in the nonrecovered trees, the embolism induced on the south side of the main axis during the winter persisted in the apical portion (Fig. 7). The only difference in the dye pattern of these embolized stems from their winter state is a narrow band of stained vessels next to the cambium on the south side; these are the functional vessels of 1987 (Fig. 7b, arrow).

DISCUSSION—Embolism occurred to a considerable degree in sugar maple saplings, even during a growing season with above average rainfall. Its preferential occurrence in the more basal segments of the trees during summer (Fig. 1A, 2A) is counter to predictions of the segmentation hypothesis (Zimmermann, 1983). If the mechanism of this embolism is strictly re-

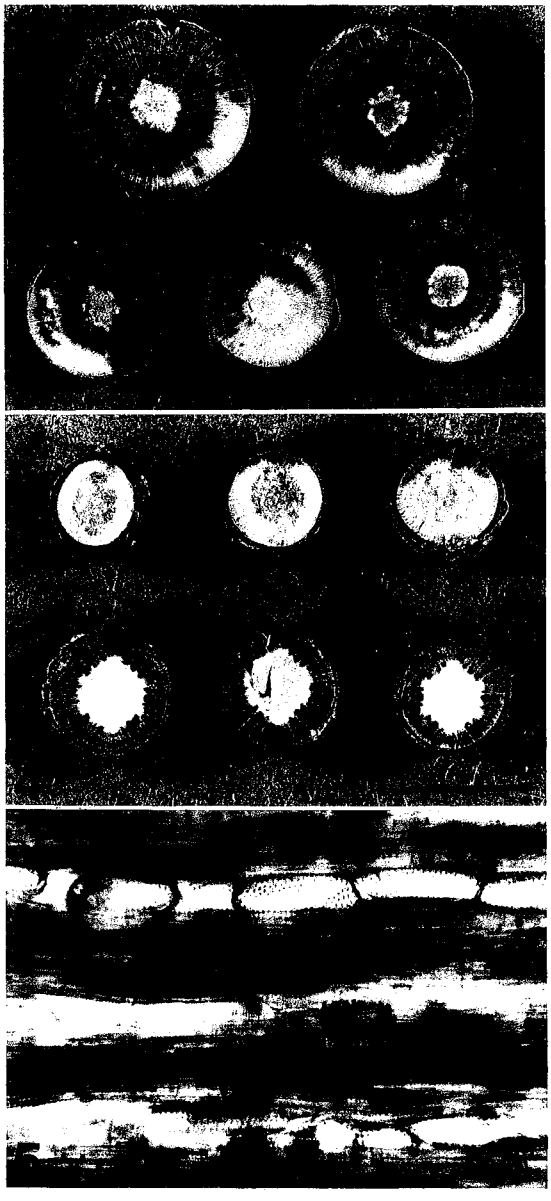

Fig. 3–5. **3.** February main axis segments from 5 trees perfused with dye. All show southern localization of nonstained, embolized xylem (notch marks north, scale bar is 0.5 cm). **4a.** February twig segments perfused with dye showing extreme proportion of nonstained, embolized xylem. **b.** Adjacent segments from same tree as **a,** but perfused with dye after having been flushed to maximum conductivity. All the xylem is stained, confirming that flushing eliminates embolism. Scale bar is 0.5 cm. **5.** Longitudinal section from partially embolized segment such as those in Fig. 3; safranin-stained vessels identified by dark-staining contact cells in black and white (arrows) are water filled, whereas the nonstained vessels contain air bubbles. Scale bar is approximately 30 μm.

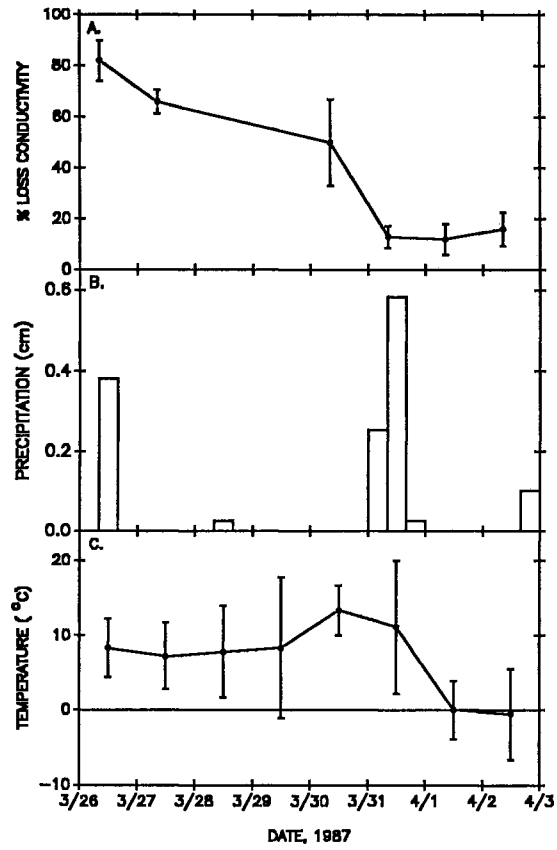

Fig. 6. **A.** Mean percentage loss in conductivity (with 95% confidence intervals) for each of the 6 trees in the March measurement vs. date. **B.** Precipitation vs. date. **C.** Mean temperature with range vs. date. (Weather data from National Weather Service Office within 3.5 km of study site.)

lated to water stress, it means xylem in the trunk is inherently more vulnerable than in twigs, despite less negative xylem pressures in the trunk. It remains to be seen what pattern of embolism would emerge under drought conditions.

In the year studied, the most severe embolism occurred during the winter with twigs averaging 84% loss of conductivity in February. What was the mechanism for winter embolism and why was it localized in the southern-facing xylem (Fig. 4a)? Freeze and thaw cycles alone could not have been responsible because we found that segments previously flushed to maximum conductivity and subsequently frozen did not lose a significant amount of conductivity relative to non-frozen controls (unpublished data); apparently bubbles formed during freezing are small and dissolve rapidly on thawing. The south side of the trunk re-

ceives the most exposure to the sun which could cause temperatures at the cambium to increase as much as 5 C above the air (Tyree, 1983, 280). Warming would enhance water loss by sublimation or evaporation depending on whether the xylem remained frozen. Either case could lead to the localized development of embolism on the southern side. In the frozen state emboli formed during freezing could grow as ice was lost to sublimation and become large enough to remain after thawing. If water was lost by evaporation during a local thaw on the south side of a trunk and uptake was prevented by frozen soil or a completely frozen root collar, negative pressures would develop. A subsequent freeze-thaw cycle could cause stable emboli to form because negative pressure would tend to expand the bubbles formed by freezing. Preliminary pressure-bomb measurements of thawed twig tips averaged -0.44 ± 0.131 MPa ($N = 6$) in January, indicating that negative pressure could exist during a partial thaw in the absence of water uptake.

These mechanisms would explain the increase in embolism with decreasing diameter in midwinter (Fig. 2B), since both exposure to the sun, and the surface-to-volume ratio of the stem would increase apically in a tree. In addition, the increase in embolism in February coincided not only with very low temperatures, but with unusually sunny weather (February 1987 was the sunniest month on record in Burlington) which would have led to greater warming and water loss from the southern sides of trunks.

The sugar maples we studied recovered nearly all of their conductivity by the beginning of the growing season (Fig. 1A). What was the mechanism for embolism recovery in these trees? It is not simply the generation of new vessels since dye perfusions indicated staining of all vessels in recovered trees (Fig. 7a, left). In addition, recovery began in March before vessels were differentiated, and some trees were not as fully recovered as others in May and June well after vessels were being produced (Fig. 2C, D; Fig. 7a, right).

During the March measurement period when recovery was apparently in progress (Fig. 6) we observed bleeding of xylem sap from wounds in the trees, and measured positive pressures of at least 16 kPa with manometers attached to lateral branch stubs. It is very likely that these pressures were dissolving the air emboli and causing the observed recovery. These pressures may have originated in the roots because air temperatures during the recovery period (Fig. 6C) were generally above freezing and may not have activated the stem-flow mechanism typical of *Acer* species. In addition, each decrease in percentage loss (Fig. 6A) is associated with rainfall (Fig. 6B) which would have favored development of root pressure.

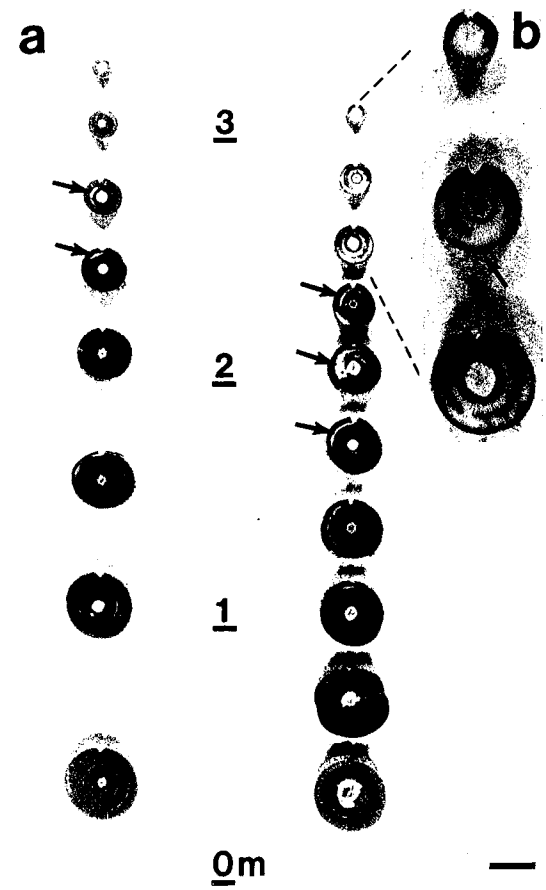

Fig. 7. **a.** Dye perfusions of main axis segments in May 1987 vs. height for a "recovered" (see Fig. 2D) tree (left) and an "embolized" tree (right). Notches indicate north, arrows mark hail-damaged xylem, and scale bar is 1 cm. The embolized tree on the right shows winter pattern of south-facing embolism (Fig. 3) persisting in upper main axis; this accounts for increasing loss of conductivity with decreasing diameter shown in Fig. 2D for "embolized" trees. **b.** Magnified view of upper 3 segments of embolized tree showing south-facing embolism and outer ring of functional xylem produced in spring 1987 (arrow).

There is no doubt that root pressure occurs in sugar maples; during the wet summer of 1986 pressures of up to 59 kPa were measured with manometers attached to stumps of recently cut trees; intact trees showed no positive pressure, probably because of transpiration. Again in October following leaf drop root pressure was observed; presumably any influence of these pressures on dissolving air bubbles was counteracted by the subfreezing weather in No-

vember. If our supposition that root pressure was chiefly responsible for embolism recovery is correct, the abnormally dry spring of 1987 (Fig. 1C) may explain why, on the average, trees did not fully return to the low embolism level of May and June 1986.

Although root pressure may have been more important than stem pressure in embolism recovery during spring 1987, there is no reason to believe that stem pressure associated with freeze-thaw cycles extensively documented for sugar maple (e.g., Marvin and Greene, 1951) could not act in a similar way. In fact, for tall trees, stem pressure may be the only mechanism that can produce positive pressure high in the canopy.

If there had been no recovery from February embolism levels, we suspect growth in these trees would have declined. Root and/or stem pressures responsible for recovery are thus essential for the health of these trees; these positive pressure mechanisms are not the insignificant curiosities they are often portrayed to be. The fact that embolism may pose a significant threat to tree growth means that how well a tree copes with the problem in a given habitat may determine its competitive success, and ultimately its ecological range. In view of this, embolism may be an important factor in the ecophysiology of trees.

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